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(57) Abstract

A process for simultaneously desizing and bleaching of a sized fabric containing starch or starch derivatives, which process comprises treating the fabric with a bleaching composition and an oxidation stable α-amylase.

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THE USE OF AN ALFA-AMYLASE MODIFIED TO IMPROVE OXIDATION STABILITY IN A COMBINED DESIZING AND BLEACHING PROCESS

FIELD OF THE INVENTION

The present invention relates to a process for simultaneously desizing and bleaching of a fabric comprising starch or starch-derivatives as well as to the use of an oxidation stable α-amylase for said process.

BACKGROUND OF THE INVENTION

In the textile processing industry, α-amylases are traditionally used as auxiliaries in the desizing process to facilitate the removal of starch-containing size which has served as a protective coating on yarns during weaving.

Complete removal of the size coating after weaving is important to ensure optimum results in the subsequent processes, in which the fabric is generally scoured, bleached and dyed. Enzymatic starch break-down is preferred because it does not involve any harmful effect on the fibre material.

In order to reduce processing cost and increase mill throughput, the desizing processing is sometimes combined with the scouring and bleaching steps. In such cases, non-enzymatic auxiliaries such as alkali or oxidation agents are typically used to break down the starch, because traditional α-amylases are not very compatible with high pH levels and bleaching agents. Alternatively, unrealistic high amounts of α-amylases, optionally in protected form, have to be used for such combined processes. The non-enzymatic breakdown of the starch size does lead to some fibre damage because of the rather aggressive chemicals used.

Accordingly, it would be desirable to use α-amylase enzymes having an improved resistance towards or being

compatible with oxidation (bleaching) agents at elevated pH, in order to retain the advantages of enzymatic size break down in a time-saving and environmentally desirable simultaneous desizing and bleaching process.

US 4,643,736 discloses a process for desizing and bleaching performed in a single operation, in which sodium chlorite is used in combination with a strong base, a surface active agent, an activator, and an amylolytic enzyme. However, the use of sodium chlorite is undesirable from an environmental point of view.

EP 119 920 discloses a process for simultaneous desizing and bleaching, in which sodium tetraborate decahydrate is used as buffer in a bath containing hydrogen peroxide, a sequestering agent, an amylase and a surfactant.

In both of the processes described in the above patent publications a relatively high amount of α-amylase is used, presumably in order to compensate for the low oxidation stability of the α-amylases used.

PCT/DK93/00230 discloses α-amylase mutants having improved oxidation stability. The mutants are indicated to be useful for desizing, but their use in a combined desizing and bleaching process is not mentioned.

BRIEF DISCLOSURE OF THE INVENTION

The fact that oxidation stable α-amylases now, for the first time, are available makes it possible to perform a combined enzymatic desizing and bleaching treatment of starch-containing fabrics. Accordingly, in a first aspect the invention relates to a process for simultaneously desizing and bleaching of a sized fabric containing starch or starch derivatives, which process comprises treating

the fabric with a bleaching composition and an oxidation stable α-amylase.

It is contemplated that the use of oxidation stable α-amylases in the above process constitute an environmentally desirable alternative to non-enzymatic alkali or oxidation agents used today for desizing and bleaching. Furthermore, the oxidation stable α-amylase may be used in amounts corresponding to the amounts of α-amylase used in today's desizing processes.

In the present context, the term "oxidation stable" is intended to indicate that under conditions prevailing during the combined process of the invention, the oxidation stable α-amylase performs better than the *B. licheniformis* α-amylase, commercially available from the Applicant under the trade name Termamyl®. Termamyl® is presently considered to be highly useful for desizing, but is less suitable for a combined process due to a relatively low tolerance towards bleaching agents normally used for bleaching. The better performance may, e.g., be measured as described in the section entitled "Determination of oxidation stability" hereinafter.

The term "desizing" is intended to be understood in a conventional manner, i.e. the removal of size from the fabric, the term "scouring" the removal of non-cellulosic materials such as grease, wax, protein, hemi-cellulosic material, pectin, ash, dirt and oil, and term "bleaching" the bleaching of coloured impurities associated with the fibers of the fabric.

The term "simultaneously" is intended to indicate that the desizing and bleaching are carried out in a single operation. This has the obvious advantage that the washing and other treatments normally performed between separately conducted desizing and bleaching steps are no longer required. Thereby, the water and energy demand as well as

the demand to different equipment to be used for each of the processes are considerably reduced. Furthermore, depending on the type of fabric to be treated and the nature of impurities present thereon, a scouring effect may be obtained during the performance of the process of the invention. Thus, in such cases, no additional scouring treatment need to be performed.

The term "fabric containing starch or starch derivatives" is intended to indicate any type of fabric, in particular woven fabric prepared from a cellulose-containing material, containing starch or starch derivatives. The fabric is normally made of cotton, viscose, flax and the like. The main part of the starch or starch derivatives present on the fabric is normally size with which the yarns, normally warp yarns, have been coated prior to weaving. In the present context, the term "fabric" is also intended to include garments and other types of processed fabrics.

In a second aspect the invention relates to a composition to be used in a simultaneous desizing and bleaching process, which composition comprises an oxidation stable α-amylase in combination with at least one further component selected from the group consisting of wetting agents, dispersing agents, sequestering agents and emulsifying agents.

In a final aspect the invention relates to the use of an oxidation stable α-amylase for a simultaneous desizing and bleaching process.

DETAILED DISCLOSURE OF THE INVENTION

<u>The oxidation stable α-amylase</u>

A preferred example of an α-amylase to be used in the process of the invention is one which has been prepared

from a parent α-amylase by replacing one or more methionine residues of the parent α-amylase with any amino acid residue different from Cys or Met. Thus, according to the invention the amino acid residues to replace the methionine amino acid residue are the following: Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, and Val.

It has surprisingly been found that the mutant α-amylases prepared as described above exhibit a better activity level and a better stability in the presence of oxidizing agents than prior art mutant amylases.

It is preferred that the oxidation stable α-amylase to be used in the present process is of microbial origin. More particularly, it is preferred that the α-amylase is derivable from a strain of *Bacillus*. Thus, *Bacillus* α-amylases exhibit in themselves a high heat stability, and by being mutated as described above, the mutants may exhibit an even better stability, especially in the presence of oxidizing agents.

In the present context the term "derivable" is intended not only to indicate an α-amylase produced by a strain of the organism in question, but also an α-amylase encoded by a DNA sequence isolated from such strain and produced in a host organism transformed with said DNA sequence. Furthermore, the term is intended to indicate an α-amylase which is encoded by a DNA sequence of synthetic and/or cDNA origin and which has the identifying characteristics of the α-amylase in question.

Examples of parent *Bacillus* α-amylases useful for the present purpose are those derivable from a strain of *B. licheniformis*, a strain of *B. amyloliquefaciens*, or a strain of *B. stearothermophilus*.

The amino acid sequence for a *B. licheniformis* α-amylase useful for the present purpose is apparent from SEQ ID No. 2 (the corresponding DNA sequence is shown in SEQ ID No. 1). G. L. Gray et al., J. Bacteriol. 166, 635-643, 1986, FR 2665178 and EP 410 498 disclose variants of said α-amylase. The methionine numbers of the *B. licheniformis* α-amylase are: 8, 15, 197, 256, 304, 366, and 438.

The amino acid sequence for a *B. amyloliquefaciens* α-amylase useful for the present purpose is apparent from SEQ ID No. 4 (the corresponding DNA sequence is shown in SEQ ID No. 3). Takkinen et al., J. Biol. Chem. 258, 1007-1013, 1983 discloses a variant of said α-amylase. The methionine numbers of these *B. amyloliquefaciens* α-amylases are: 6, 197, 256, 304, 366, and 438.

The amino acid sequence for a *B. stearothermophilus* α-amylase useful for the present purpose is apparent from SEQ ID No. 6 (the corresponding DNA sequence is shown in SEQ ID No. 5). G.L. Gray et al., J. Bacteriol. 166, 635-643, 1986 disclose a variant of said α-amylase. The methionine numbers of these *B. stearothermophilus* α-amylases are: 8, 9, 97, 200, 206, 284, 307, 311, 316, and 437.

Furthermore, a parent α-amylase of fungal origin may be used, e.g. an α-amylase derivable from a strain of the fungal genus *Aspergillus*. For instance, the parent α-amylase may be derivable from a strain of the fungal species *A. oryzae* or *A. niger*. These α-amylases are all well characterized and their entire amino acid sequence is described.

The amino acid sequence for the *Asp. oryzae* α-amylase (sold commercially as FUNGAMYL®, by Novo Nordisk A/S) is shown in SEQ ID No. 7. The amino acid sequence of an *A. niger* α-amylase is shown in DK 5126/87.

In a preferred embodiment the parent α-amylase is selected from the group consisting of a *B. licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus*, *A. oryzae* and *A. niger* α-amylase, or is a functional analogue of any of said parent α-amylases which

i) comprises an amino acid sequence being at least 60% homologous with the amino acid sequence of the parent α-amylase,

ii) reacts with an antibody raised against the parent α-amylase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding the parent α-amylase.

Property i) of the analogue is intended to indicate the degree of identity between the analogue and the parent α-amylase indicating a derivation of the first sequence from the second. In particular, a polypeptide is considered to be homologous to the parent α-amylase if a comparison of the respective amino acid sequences reveals an identity of greater than about 60%, such as above 70%, 80%, 85%, 90% or even 95%. Sequence comparisons can be performed via known algorithms, such as the one described by Lipman and Pearson (1985).

The homologous α-amylase may be a genetically engineered α-amylase, e.g. prepared in order to improve one or more properties such as thermostability, acid/alkaline stability, temperature or pH optimum and the like.

The additional properties ii) and iii) of the analogue of the parent α-amylase may be determined as follows:

Property ii), i.e. the immunological cross reactivity, may be assayed using an antibody raised against or reactive

with at least one epitope of the parent α-amylase. The antibody, which may either be monoclonal or polyclonal, may be produced by methods known in the art, e.g. as described by Hudson et al., 1989. The immunological cross-reactivity may be determined using assays known in the art, examples of which are Western Blotting or radial immunodiffusion assay, e.g. as described by Hudson et al., 1989. In this respect, immunological cross-reactivity between parent *B. licheniformis*, *B. amyloliquefaciens* and *B. stearothermophilus* α-amylases having the amino acid sequences SEQ ID Nos. 2, 4 and 6, respectively, have been found.

The oligonucleotide probe used in the characterization of the analogue in accordance with property iii) defined above, may suitably be prepared on the basis of the full or partial nucleotide or amino acid sequence of the parent α-amylase. The hybridization may be carried out under any suitable conditions allowing the DNA sequences to hybridize. For instance, such conditions are hybridization under specified conditions, e.g. involving presoaking in 5xSSC and prehybridizing for 1h at ~40°C in a solution of 20% formamide, 5xDenhardt's solution, 50mM sodium phosphate, pH 6.8, and 50μg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100μM ATP for 18h at ~40°C, or other methods described by e.g. Sambrook et al., 1989.

Specific examples of analogues of the *B. licheniformis* α-amylase comprising the amino acid sequence shown in SEQ ID No. 2 are Termamyl® (available from Novo Nordisk A/S), Optitherm® and Takatherm® (available from Solvay), Maxamyl® (available from Gist-Brocades), Spezym AA® (available from Genencor), and Keistase® (available from Daiwa).

Specific examples of analogues of the *B. amyloliquefaciens* α-amylase comprising the amino acid sequence shown in SEQ

ID No. 4 are BAN® (available from Novo Nordisk A/S), Optiamyl® (available from Solvay), Dexlo® and Rapidase® (available from Gist-Brocades), Kazuzase® (a mixed α-amylase and protease product available from Showa Denko).

Specific examples of analogues of the *B. stearothermophilus* α-amylase comprising the amino acid sequence shown in SEQ ID No. 6 are Liquozyme 280L® (available from Novo Nordisk A/S) and G-zyme 995® (available from Enzyme BioSystems).

In a preferred embodiment of the process of the invention the oxidation stable α-amylase is prepared from a parent α-amylase by replacing one or more of the methionine amino acid residues with a Leu, Thr, Ala, Gly, Ser, Ile, Asn, or Asp amino acid residue, preferably a Leu, Thr, Ala, or Gly amino acid residue.

In the present context, a mutant α-amylase of particular interest is one, in which the methionine amino acid residue in position 197 in *B. licheniformis* α-amylase or the methionine amino acid residue in homologous positions in other α-amylases is exchanged. The concept of homologous positions or sequence homology of α-amylases has been explained e.g. in Nakajima, R. et al., 1986, Appl. Microbiol. Biotechnol. 23, 355-360 and Liisa Holm et al., 1990, Protein Engineering 3, 181-191. Sequence homology of *Bacillus* α-amylases from *B. licheniformis*, *B. stearothermophilus* and *B. amyloliquefaciens* are about 60%. This makes it possible to align the sequences in order to compare residues at homologous positions in the sequence. By such alignment of α-amylase sequences the number in each α-amylase sequence of the homologous residues can be found. The homologous positions will probably spatially be in the same position in a three dimensional structure (Greer, J., 1981, J. Mol. Biol. 153, 1027-1042), thus having analogous impact on specific functions of the enzyme in question. In relation to position 197 in *B. licheniformis* α-amylase the homologous positions in *B.*

stearothermophilus α-amylase are positions 200 and 206, and the homologous position in *B. amyloliquefaciens* α-amylase is position 197. Experimentally it has been found that these mutants exhibit both an improved activity level and an improved stability in the presence of oxidizing agents.

Accordingly, another type of oxidation stable α-amylases of interest for the present purpose, is an oxidation stable α-amylase prepared by replacing one or both of the methionine amino acid residues in positions 200 and 206 in a parent *B. stearothermophilus* α-amylase or the methionine amino acid residues in homologous positions in other α-amylases with other amino acid residues as explained above.

The mutant α-amylases disclosed above may be constructed in accordance with established methods, e.g. by use of site-directed mutagenesis.

<u>Process conditions</u>

It will be understood that the combined process of the invention may be performed in accordance with any suitable desizing or bleaching process known in the art, e.g. as described by Olson, E.S. "Textile Wet Processes, Vol. I, Noyes Publication, Park Ridge, New Jersey, USA (1983), M. Peter und H.K Rouette, Grundlagen der Textilveredlung, Deutsche Fachverlag GmbH, Frankfurt am Main, Germany (1988). Thus, the process conditions to be used in performing the present invention may be selected so as to match a particular equipment or a particular type of process which it is desirable to use. Preferred examples of process types to be used in connection with the present invention include Jigger/Winch, Pad-Roll and Pad-Steam types. These types are dealt with in further detail below.

The combined process of the invention may be carried out as a batch, semi-continuous or continuous process using

steam or the principles of cold-bleaching. As an example the process may comprise the following steps:

(a) Impregnating the fabric in a desizing and bleaching bath containing (as a minimum) an oxidation stable α-amylase and a bleaching agent followed by squeezing out excessive liquid so as to maintain the quantity of liquor necessary for the reaction to be carried out (normally between 60 and 120% of the weight of the dry fabric),

(b) subjecting the impregnated fabric to steaming so as to bring the fabric to the desired reaction temperature, generally between 20° and 120°C, and

(c) holding by rolling up or pleating the cloth in a J-Box, U-Box, carpet machine or the like for a sufficient period of time (normally between a few minutes and several hours) to allow the desizing and bleaching to occur.

As mentioned above, scouring may be an inherent result obtained when performing the combined process of the invention. However, for certain types of fabric it may be advantageous and/or necessary to subject the fabric to a scouring treatment in order to obtain a final product of a desired quality. In such cases, oxidation stable α-amylases disclosed herein may be employed in a combined desizing and scouring process, in particular oxidation stable α-amylases which are sufficiently stable at the high pH values, at which scouring is normally performed. Typically, a combined desizing and scouring process is carried out using a sufficient amount of an oxidation stable α-amylase and a strong alkali, such as NaOH, under conditions known in the art for desizing and scouring to be performed. Subsequently, the fabric resulting from a combined desizing and scouring treatment may be subjected to bleaching.

Normally, the oxidation stable α-amylase and the bleaching composition are added separately to the equipment in which the combined process is to take place. However, the oxidation stable α-amylase may also be mixed with the bleaching composition immediately prior to the combined treatment to be performed.

Although any type of bleaching agent may be used (such as sodium chlorite, sodium hypo chlorit and hydrogen peroxide) in the process of the invention it is preferred that a hydrogen peroxide based bleaching composition is used. Hydrogen peroxide constitutes the most gentle and environmentally friendly bleaching agent available today. The hydrogen peroxide is normally used in the form of a 35% solution and in an amount of 1-50 g/l of bleaching bath liquid, such as in an amount of 5-40 g/l, 5-30 g/l, 10-30 g/l or 30-40 g/l depending on the type of process to be used.

Further components required for the process to be performed are typically added separately. Examples of such components include a stabilizer and a wetting agent. The stabilizer may be an agent stabilizing the hydrogen peroxide (such as water glass ($Na2O:SiO2$) and a Magnesium salt) so as to control the reactivity of the hydrogen peroxide.

The wetting agent serves to improve the wettability of the fibre whereby a rapid and even desizing and bleaching may be obtained. The wetting agent is preferably of an oxidation stable type.

In a preferred embodiment of the process of the invention, the oxidation stable α-amylase is used in an amount exceeding 1 g/l, preferably in an amount of 1-20 g/l, such as 1-10 g/l, 1-5 g/l or 1-3 g/l. It will be understod that the amount of α-amylase to be used depend on the formulation of the α-amylase product in question.

Irrespective of the particular type of process to be used for the combined desizing and bleaching of the invention, the combined process is normally performed at a temperature in the range of 30-100°C, such as 50-100°C, 80-100°C, 90-100°C or 90-95°C and a pH in the range of 6.5-11, such as 9-10.8 or 10.0-10.8 . However, the actual process conditions may vary widely within these ranges as will be apparent from the following disclosure.

Preferred examples of the process conditions to be used in connection with the present invention include:

A batch type process, e.g. of the Jigger/Winch type, using

1-5 g/l of an oxidation stable α-amylase,
6-25 g/l of hydrogen peroxide (35%),
7-14 g/l of stabilizer, e.g. water glass,
0.25-5 g/l of a wetting agent, e.g. Arbyl R, available from Grünau, Germany,
the process being performed at a pH in the range of 10-11 (obtained by addition of NaOH) and a temperature in the range of 90-95°C (obtained by steaming), typically for 1-2 hours.

A semi-continuous process, e.g. of the Pad-Roll type, using
1-5 g/l of an oxidation stable α-amylase,
30-40 g/l of hydrogen peroxide (35%),
12-30 g/l of stabilizer, e.g. water glass,
0.25-5 g/l of a wetting agent, e.g. Arbyl R,
the process being performed at a pH in the range of 10-11 (obtained by addition of NaOH) and a temperature in the range of 20-40°C, typically for 12-24 hours.

A continuous process, e.g. of the Pad-Steam type, using
1-5 g/l of an oxidation stable α-amylase,
8-25 g/l of hydrogen peroxide (35%),
5-20 g/l of stabilizer, e.g. water glass,

0.25 - 5 g/l of a wetting agent, e.g. Arbyl R,
the process being performed at a pH in the range of 10-11 (obtained by addition of NaOH) and a temperature in the range of 98-140°C (steaming), the temperature above 115 preferably being maintained for a few seconds only and the process time typically being 0.5-3 min.

It will be understood that the combined process may be performed in any equipment sufficiently tolerant towards the conditions of the process.

Furthermore, it will be evident that in addition to the oxidation stability of the α-amylase to be used in the process of the invention this amylase should preferably be one which is active at a pH of above 6.5, such as above 9.0. Preferably the oxidation stable α-amylase has a high activity in the pH range of 10-10.8.

<u>Composition of the invention</u>

Although the oxidation stable α-amylase may be added as such it is preferred that it is formulated into a suitable composition. Thus, the oxidation stable α-amylase may be used in the form of a granulate, preferably a non-dusting granulate, a liquid, in particular a stabilized liquid, a slurry, or in a protected form. Dust free granulates may be produced, e.g. as disclosed in US 4,106,991 and US 4,661,452 (both to Novo Nordisk A/S) and may optionally be coated by methods known in the art.

Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as e.g. propylene glycol, a sugar or sugar alcohol or acetic acid, according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238 216.

In principle the composition of the invention comprising an oxidation stable α-amylase may contain any other agent

to be used in the combined process of the invention. However, it is preferred that the composition is free from the bleaching agent and other highly oxidizing agents.

The composition of the invention comprises at least one further component selected from the group consisting of wetting agents, dispersing agents, sequestering agents and emulsifying agents. Examples of suitable wetting agents are disclosed above. The emulsifying agent serves to emulsify hydrophobic impurities present on the fabric. The dispersing agent serves to prevent that extracted impurities redeposit on the fabric. The sequestering agent serve to remove ions such as Ca, Mg and Fe, which may have a negative impact on the process and preferred examples include caustic soda (sodium hydroxide) and soda ash (sodium carbonate).

Determination of oxidation stability

The amylase preparation is diluted to an amylase activity of 100 NU/ml (the NU (or KNU which is 1000 NU) amylase activity assay is defined in AF 207/1, which is available on request from Novo Nordisk A/S, and the unit is defined as follows: 1 KNU is the amount of enzyme which, per hour, under standard conditions, dextrinized 5.26 g starch dry substance Merch Amylum solubile, cat. no. 1253) in 50 mM of a Britton-Robinson buffer at pH 6-10 and incubated at 40-90°C. Subsequently H_2O_2 (35%) is added to a concentration of 1-50 g/l, and the pH value is readjusted to the desired value. the activity is now measured after 15 seconds and after 5, 15, and 30 minutes, the activities are determined as described in AF 207/1 but with a standard curve constructed at the chosen pH and temperature instead of at pH 7.3, 37°C. the results are compared to measurements of a preparation of Termamyl® (available from Novo Nordisk A/S, Denmark) under the same conditions.

The following non-limiting examples illustrates the invention.

EXAMPLE 1

Comparison of the pH-profile of oxidation stable α-amylase and conventional amylase

Materials and methods:

Textile:

100% pure cotton, unbleached, 5cm x 5cm swatches.

Enzymes:

A: *Bacillus licheniformis* oxidation stable α-amylase (methionin 197 replaced with leucin, activity 6.2 KNU_p/g, prepared by the Applicant, batch A943043K).

B: *Bacillus licheniformis* α-amylase (Termamyl 120L, activity 142 KNU_p/g, commercially available from the Applicant, batch AXR 4025 94-3).

Dosage: 30 KNU_p enzyme/100ml buffer.

Buffer: Britton-Robinson, pH 7 to 11
Dosage: 100ml buffer/shake flask

100 ml buffer was added to a shake flask and placed in a heated water bath at 85°C. When the buffer had reached the temperature of 85°C, the enzyme preparation (A or B, respectively) was added together with one swatch. The swatches were treated in a shaking waterbath for 20 minutes at 85°C.

After the enzyme treatment the swatches were washed at 95°C in 100 ml water containing 2g of Kieralon CD/litre (a surfactant), followed by rinsing 4 times, each time in 100 ml cold deionized water. Then, the swatches were dried at

105°C for 10 minutes. After drying the swatches were tested using the conventional TEGEWA method (Method and standard scales obtainable from Verband TEGEWA, Karlstrasse 21, Frankfurt a.M., Germany).

TEGEWA method:
The samples of desized fabric were impregnated in a saturated iodine solution (5 ml/100 ml water), rinsed with cold water, and wiped off with filter paper before comparing the (blue) colour intensity with the TEGEWA violet standard scale.
The TEGEWA standard scale ranges from 1 to 9, where 1 is the sized fabric while 9 is the totally desized fabric. A rating above 6 usually corresponds to an acceptable desizing.

The results from the pH experiments are shown in table 1 below..

Table 1

pH	**Reference**	**Enzyme B** (conventional)	**Enzyme A** (ox. stable)
7	1	6	7
8	1	5	7
9	1	4	7
10	1	2	5

The results show that the oxidation stable A-amylase is more pH-stable than the conventional A-amylase (Termamyl).

EXAMPLE 2

The oxidation stable α-amylase was tested and compared with a conventional amylase (Termamyl) in a combined process of desizing/bleaching.

Materials and methods:

As described in example 1, and further:

H_2O_2 concentration:	4, 6, 8, 10, 24 g/l, respectively
NaOH conc.:	0.5, 1.0 g/l, respectively
Stabilizer:	2 g/l (Product No. 1136 from the company Harald Pedersen, Kastanievej 7, 1878 Frb.C, Denmark)
Process temp.:	80°C, 85°C, 95°C, respectively
Process time:	60 minutes (20 min. for temp. 85°C and 95°C)

The desizing/bleaching solution was preheated to 80°C (85°C, 95°C) in a shake flask, the swatches were added to the solution, and the shake flask was placed in a shaking water bath for 1 hour. The concentration of H_2O_2 in the solution was monitored by titration immediately before use. The washing/rinsing and evaluation of the treated swatches was as described in example 1.

The results from the desizing/bleaching experiments are shown in table 2 below.

Table 2

H_2O_2 g/l	Temp. °C	NaOH g/l	pH	Reference score	Enzyme B score	Enzyme A score
4	80	0.5	10.1	2	5	7
6	80	0.5	9.9	1	3	6
6	80	1.0	10.1	2	3	5
8	80	1.0	10.2	2	3	5
10	85	0.5	9.6	2	4	5
10	95	0.5	9.5	3	5	6
24	85	0.5	9.1	3	4	6
24	95	0.5	8.7	4	5	7
10	95	1.0	10.0	[illegible]	4	5

The results demonstrate that the oxidation stable A-amylase is more stable at high pH and at high H_2O_2 concentrations than the conventional amylase (Termamyl).

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M. Peter und H.K Rouette, Grundlagen der Textilveredlung, Deutsche Fachverlag GmbH, Frankfurt am Main, Germany (1988);

SEQUENCE LISTING

In the following SEQ ID Nos. 1, 3, 5 the 5′, coding sequence and 3′ sequence of the relevant α-amylase genes are illustrated. The 5′ sequence is the first separate part of the sequence written with lower case letters, the coding sequence is the intermediate part of the sequence, where the signal sequence is written with lower case letters and the sequence encoding the mature α-amylase is written with upper case letters, and the 3′ sequence is the third separate part of the sequence written with lower case letters.

<u>SEQ ID No. 1</u>

cggaagattggaagtacaaaaataagcaaaagattgtcaatcatgtcatgagccatgc
gggagacggaaaaatcgtctta atgcacgatattt-
atgcaacgttcgcagatgctgctgaagagattattaaaagctgaaa-
gcaaaaggctatcaattggt aactgtatctcagcttgaagaagtgaagaag-
cagagaggctattgaataaatgagtagaagcgccatatcggcgcttttc tttt-
ggaagaaaatatagggaaaatggtacttgttaaaattcggaatatttatac-
aacatcatatgtttcacattgaaa ggggaggagaatc

atgaaacaacaaaaacggctttacgcccgattgctgacgctgttatttgcgctcatct
tcttgctgc ctcattctgcagcagcggcgGCAAATCTTAATGGGACG-
CTGATGCAGTATTTTGAATGGTACATGCCCAATGACGGCCAA CAT-
TGGAGGCGTTTGCAAAACGACTCGGCATATTTGGCTGAACAC GTAT-
TACTGCCGTCTGGATTCCCCCGGCATATAA GGGAACGAG-
CCAAGCGGATGTGGGCTACGGTGCTTACGACCTTTATGATTTAGGGGAGTTTCATCAA
AAAGGGACGGTTC
GGACAAAGTACGGCACAAAAGGAGAGCTGCAATCTGCGATCAAAAGT-
CTTCATTCCCGCGACATTAACGTTTACGGGGAT
GTGGTCATCAACCACAAAGGCGGCGC-
TGATGCGACCGAAGATGTAACCGCGGTTGAAGTCGATCCCGCTGACCGCAACCG
CGTAATTTCAGGAGAACACCTAATTAAAGCCTGGACACATTT-
TCATTTTCCGGGGCGCGGCAGCACATACAGCGATTTTA AATGGCAT-
TGGTACCATTTTGACGGAACCGATTGGGACGAGTCCCGAAAGCTGAAC-
CGCATCTATAAGTTTCAAGGAAAG GCTTGGGATTGGGAAGTTTCCAATGAA-
AACGGCAACTATGATTATTTGATGTATGCCGACATCGATTATGACCATCCTGA

TGTCGCAGCAGAAATTAAGAGATGGGGCACTTGGTATGCCAATGAACTGCAAT-
TGGACGGTTTCCGTCTTGATGCTGTCA
AACACATTAAATTTTCTTTTTTGCGGGATTGGGTTAATC-
ATGTCAGGGAAAAAACGGGGAAGGAAATGTTTACGGTAGCT GAATATTGGCAGAAT-
GACTTGGGCGCGCTGGAAAACTATTTGAACAAAACAAATTTTAATCATTCAGTGTTTG
ACGTGCC GCTTCATTATCAGTTCCATGCTGCATCGACACAG-
GGAGGCGGCTATGATATGAGGAAATTGCTGAACGGTACGGTCGTTT CCAAG-
CATCCGTTGAAATCGGTTACATTTGTCGATAACCATGATACACAGCCGGGGCAA-
TCGCTTGAGTCGACTGTCCAA ACATGGTTTAAGCCGCTTGCTTACGCTTTTAT-
TCTCACAAGGGAATCTGGATACCCTCAGGTTTTCTACGGGGATATGTA CGGGA-
CGAAAGGAGACTCCCAGCGCGAAATTCCTGCCTTGAAACACAAAATTGAACCGA-
TCTTAAAAGCGAGAAAACAGT ATGCGTACGGAGCACAGCATGATTATTTCGAC-
CACCATGACATTGTCGGCTGGACAAGGGAAGGCGACAGCTCGGTTGCA AATTCAGG-
TTTGGCGGCATTAATAACAGACGGACCCGGTGGGGCAAAGCGAATGTATGTCGGCCGG
CAAAACGCCGGTGA GACATGGCATGAC-
ATTACCGGAAACCGTTCGGAGCCGGTTGTCATCAATTCGGAAG-
GCTGGGGAGAGTTTCACGTAAACG GCGGGTCGGTTTCAATTTATGTTCAAAGATAG

aagagcagagaggacggatttcctgaaggaaatccgtttttttatttt

<u>SEQ ID No. 2</u>

ANLNGTLMQYFEWYMPNDGQHWRRLQNDSAYLAEHGITAV
WIPPAYKGTSQADVGYGAYDLYDLGEFHQKGTVRTKYGTK
GELQSAIKSLHSRDINVYGDVVINHKGGADATEDVTAVEV
DPADRNRVISGEHLIKAWTHFHFPGRGSTYSDFKWHWYHF
DGTDWDESRKLNRIYKFQGKAWDWEVSNENGNYDYLMYAD
IDYDHPDVAAEIKRWGTWYANELQLDGFRLDAVKHIKFSF
LRDWVNHVREKTGKEMFTVAEYWQNDLGALENYLNKTNFN
HSVFDVPLHYQFHAASTQGGGYDMRKLLNGTVVSKHPLKS
VTFVDNHDTQPGQSLESTVQTWFKPLAYAFILTRESGYPQ
VFYGDMYGTKGDSQREIPALKHKIEPILKARKQYAYGAQH
DYFDHHDIVGWTREGDSSVANSGLAALITDGPGGAKRMYV
GRQNAGETWHDITGNRSEPVVINSEGWGEFHVNGGSVSIY
VQR

<u>SEQ ID No. 3</u>

gccccgcacatacgaaaagactggctgaaaacattgagcctttgatga-
ctgatgatttggctgaagaagtggatcgattg tttgagaaaagaaga-

agaccataaaaataccttgtctgtcatcagacagggtatttttatgctgtcca-
gactgtccgct gtgtaaaaataaggaataaagggggttgttattattttact-
gatatgtaaaatataatttgtataagaaaatgagaggg agaggaaac

atgattcaaaaacgaaagcggacagtttcgttcagacttgtgcttatgtgcacg-
ctgttatttgtcagttt gccgattacaaaaacatcagccGTAAATGGCACGC-
TGATGCAGTATTTTGAATGGTATACGCCGAACGACGGCCAGCATT GGAAAC-
GATTGCAGAATGATGCGGAACATTTATCGGATATCGGAATCACTGCCGTCTGGA-
TTCCTCCCGCATACAAAGGA TTGAGCCAATCCGATAACGGATACGGACCTTAT-
GATTTGTATGATTTAGGAGAATTCCAGCAAAAAGGGACGGTCAGAAC GAAATA-
CGGCACAAAATCAGAGCTTCAAGATGCGATCGGCTCACTGCATTCCCGGAACGT-
CCAAGTATACGGAGATGTGG TTTTGAATCATAAGGCTGGTGCTGATGCAACAG-
AAGATGTAACTGCCGTCGAAGTCAATCCGGCCAATAGAAATCAGGAA ACTTCG-
GAGGAATATCAAATCAAAGCGTGGACGGATTTTCGTTTTCCGGGCCGTGGAAAC-
ACGTACAGTGATTTTAAATG GCATTGGTATCATTTCGACGGAGCGGACTGGGA-
TGAATCCCGGAAGATCAGCCGCATCTTTAAGTTTCGTGGGGAAGGAA AAGCGT-
GGGATTGGGAAGTATCAAGTGAAAACGGCAACTATGACTATTTAATGTATGCTG-
ATGTTGACTACGACCACCCT GATGTCGTGGCAGAGACAAAAAAATGGGGTATC-
TGGTATGCGAATGAACTGTCATTAGACGGCTTCCGTATTGATGCCGC CAAACA-
TATTAAATTTTCATTTCTGCGTGATTGGGTTCAGGCGGTCAGACAGGCGACGGG-
AAAAGAAATGTTTACGGTTG CGGAGTATTGGCAGAATAATGCCGGGAAACTCG-
AAAACTACTTGAATAAAACAAGCTTTAATCAATCCGTGTTTGATGTT CCGCTT-
CATTTCAATTTACAGGCGGCTTCCTCACAAGGAGGCGGATATGATATGAGGCGT-
TTGCTGGACGGTACCGTTGT GTCCAGGCATCCGGAAAAGGCGGTTACATTTGT-
TGAAAATCATGACACACAGCCGGGACAGTCATTGGAATCGACAGTCC AAACTTGGT-
TTAAACCGCTTGCATACGCCTTTATTTTGACAAGAGAATCCGGTTATCCTC-
AGGTGTTCTATGGGGATATG TACGGGACAAAAGGGACATCGCCAAAGGAAATT-
CCCTCACTGAAAGATAATATAGAGCCGATTTTAAAAGCGCGTAAGGA GTACGC-
ATACGGGCCCCAGCACGATTATATTGACCACCCGGATGTGATCGGATGGACGAG-
GGAAGGTGACAGCTCCGCCG CCAAATCAGGTTTGGCCGCTTTAATCACGGACGGAC-
CCGGCGGATCAAAGCCGATGTATGCCGGCCTGAAAAATGCCGGC GAGACATGG-
TATGACATAACGGGCAACCGTTCAGATACTGTAAAAATCGGATCTGACGGCTGG-
GGAGAGTTTCATGTAAA CGATGGGTCCGTCTCCATTTATGTTCAGAAATAA

ggtaataaaaaaacacctccaagctgagtgcgggtatcagcttgga ggtgcgtt-
tattttttcagccgtatgacaaggtcggcatcaggtgtgacaaatacggtatgc-
tggctgtcataggtgaca aatccgggttttgcgccgtttggctttttcacatg-
tctgattttgtataatcaacaggcacggagccggaatctttcgc cttggaaa-

aataagcggcgatcgtagctgcttccaatatggattgttcatcgggatcgctgc-
ttttaatcacaacgtggg atcc

SEQ ID No. 4

VNGTLMQYFEWYTPNDGQHWKRLQNDAEHLSDIGITAVWI
PPAYKGLSQSDNGYGPYDLYDLGEFQQKGTVRTKYGTKSE
LQDAIGSLHSRNVQVYGDVVLNHKAGADATEDVTAVEVNP
ANRNQETSEEYQIKAWTDFRFPGRGNTYSDFKWHWYHFDG
ADWDESRKISRIFKFRGEGKAWDWEVSSENGNYDYLMYAD
VDYDHPDVVAETKKWGIWYANELSLDGFRIDAAKHIKFSF
LRDWVQAVRQATGKEMFTVAEYWQNNAGKLENYLNKTSFN
QSVFDVPLHFNLQAASSQGGGYDMRRLLDGTVVSRHPEKA
VTFVENHDTQPGQSLESTVQTWFKPLAYAFILTRESGYPQ
VFYGDMYGTKGTSPKEIPSLKDNIEPILKARKEYAYGPQH
DYIDHPDVIGWTREGDSSAAKSGLAALITDGPGGSKRMYA
GLKNAGETWYDITGNRSDTVKIGSDGWGEFHVNDGSVSIY

SEQ ID No. 5

aaattcgatattgaaaacgattacaaataaaaattataataga-
cgtaaacgttcgagggtttgctccctttttactcttt ttatgcaatcgtttcc-
cttaatttttggaagccaaaccgtcgaatgtaacatttgattaagggggaag-
ggcatt

gtgct aacgtttcaccgcatcattcgaaaggatggatgttcctgctcgcgtt-
tttgctcactgtctcgctgttctgcccaacag gacagcccgccaaggctGCCG-
CACCGTTTAACGGCACCATGATGCAGTATTTTGAATGGTACTTGCCGGATGATG-
GCACG TTATGGACCAAAGTGGCCAATGAAGCCAACAACTTATCCAG-
CCTTGGCATCACCGCTCTTTGGCTGCCGCCCGCTTACAA AGGAACAAGCCGCA-
GCGACGTAGGGTACGGAGTATACGACTTGTATGACCTCGGCGAATTCAATCAAA-
AAGGGACCGTCC GCACAAAATACGGAACAAAAGCTCAATATCTTCAAGCCAT-
TCAAGCCGCCCACGCCGCTGGAATGCAAGTGTACGCCGAT GTCGTGTTCGAC-
CATAAAGGCGGCGCTGACGGCACGGAATGGGTGGACGCCGTCGAAGTCAATCCG-
TCCGACCGCAACCA AGAAATCTCGGGCACCTATCAAATCCAAGCATGGACGAAATT-
TGATTTTCCCGGGCGGGGCAACACCTACTCCAGCTTTA AGTGGCGCTGGTACCAT-
TTTGACGGCGTTGATTGGGACGAAAGCCGAAAATTGAGCCGCATTTACAAAT-
TCCGCGGCATC GGCAAAGCGTGGGATTGGGAAGTAGACACGGAAAACGGAAAC-
TATGACTACTTAATGTATGCCGACCTTGATATGGATCA TCCCGAAGTCGTGAC-
CGAGCTGAAAAACTGGGGGAAATGGTATGTCAACACAACGAACATTGATGGGTT-

CCGGCTTGATG CCGTCAAGCATATTAAGTTCAGTTTTTTCCTGATTGGTTGT-
CGTATGTGCGTTCTCAGACTGGCAAGCCGCTATTTACC GTCGGGGAATATTGG-
AGCTATGACATCAACAAGTTGCACAATTACATTACGAAAACAGACGGAACGATG-
TCTTTGTTTGA TGCCCCGTTACACAACAAATTTTATACCGCTTCCAAATCAGG-
GGGCGCATTTGATATGCGCACGTTAATGACCAATACTC TCATGAAAGATCAAC-
CGACATTGGCCGTCACCTTCGTTGATAATCATGACACCGAACCCGGCCAAGCGC-
TGCAGTCATGG GTCGACCCATGGTTCAAACCGTTGGCTTACGCCTTTATTCTA-
ACTCGGCAGGAAGGATACCCGTGCGTCTTTTATGGTGA CTATTATGGCATTCC-
ACAATATAACATTCCTTCGCTGAAAAGCAAAATCGATCCGCTCCTCATCGCGCG-
CAGGGATTATG CTTACGGAACGCAACATGATTATCTTGATCACTCCGACATCA-
TCGGGTGGACAAGGGAAGGGGGCACTGAAAAACCAGGA TCCGGACTGGCCGCA-
CTGATCACC[illegible]ATGGGCCGGGAGGAAGCAAATGGATGTACGTTGGCAAACAACAC-
GCTGGAAAAGT GTTCTATGACCTTACCGGCAACCGGAGTGACACCGTCACCAT-
CAACAGTGATGGATGGGGGGAATTCAAAGTCAATGGCG GTTCGGTTTCGGTTT-
GGGTTCCTAGAAAAACGACCGTTTCTACCATCGCTCGGCCGATCACAACCCGAC-
CGTGGACTGGT GAATTCGTCCGTTGGACCGAACCACGGTTGGTGGCATGGCCTTGA

tgcctgcga

SEQ ID No. 6

AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITA
LWLPPAYKGTSRSDVGYGVYDLYDLGEFNQKGTVRTKYGT
KAQYLQAIQAAHAAGMQVYADVVFDHKGGADGTEWVDAVE
VNPSDRNQEISGTYQIQAWTKFDFPGRGNTYSSFKWRWYH
FDGVDWDESRKLSRIYKFRGIGKAWDWEVDTENGNYDYLM
YADLDMDHPEVVTELKNWGKWYVNTTNIDGFRLDAVKHIK
FSFFPDWLSYVRSQTGKPLFTVGEYWSYDINKLHNYITKT
DGTMSLFDAPLHNKFYTASKSGGAFDMRTLMTNTLMKDQP
TLAVTFVDNHDTEPGQALQSWVDPWFKPLAYAFILTRQEG
YPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGTQH
DYLDHSDIIGWTREGGTEKPGSGLAALITDGPGGSKWMYV
GKQHAGKVFYDLTGNRSDTVTINSDGWGEFKVNGGSVSVW
VPRKTTVSTIARPITTRPWTGEFVRWTEPRLVAW

SEQ ID No. 7

1 ATPADWRSQS IYFLLTDRFA RTDGSTTATC
31 NTADQKYCGG TWQGIIDKLD YIQGMGFTAI

```
 61 WITPVTAQLP QTTAYGDAYH GYWQQDIYSL
 91 NENYGTADDL KALSSALHER GMYLMVDVVA
121 NHMGYDGAGS SVDYSVFKPF SSQDYFHPFC
151 FIQNYEDQTQ VEDCWLGDNT VSLPDLDTTK
181 DVVKNEWYDW VGSLVSNYSI DGLRIDTVKH
211 VQKDFWPGYN KAAGVYCIGE VLDGDPAYTC
241 PYQNVMDGVL NYPIYYPLLN AFKSTSGSMD
271 DLYNMINTVK SDCPDSTLLG TFVENHDNPR
301 FASYTNDIAL AKNVAAFIIL NDGIPIIYAG
331 QEQHYAGGND PANREATWLS GYPTDSELYK
361 LIASANAIRN YAISKDTGFV TYKNWPIYKD
391 DITIAMRKGT DGSQIVTILS NKGASGDSYT
421 LSLSGAGYTA GQQLTEVIGC TTVTVGSDGN
451 VPVPMAGGLP RVLYPTEKLA GSKICSSS
```

CLAIMS

1. A process for simultaneously desizing and bleaching of a sized fabric containing starch or starch derivatives, which process comprises

treating the fabric with a bleaching composition and an oxidation stable α-amylase.

2. The process according to claim 1, in which the oxidation stable α-amylase has been prepared from a parent α-amylase by replacing one or more methionine residues of the parent α-amylase with any amino acid residue different from Cys or Met.

3. The process according to claim 1 or 2, in which the oxidation stable α-amylase is of microbial origin.

4. The process according to claim 3, in which the parent α-amylase is derivable from a strain of a *Bacillus* sp.

5. The process according to claim 4, in which the parent α-amylase is derivable from a strain of *B. licheniformis*, *B. amyloliquefaciens* or *B. stearothermophilus*.

6. The process according to claim 5, in which the parent α-amylase comprises the amino acid sequence of the *B. licheniformis* α-amylase shown in SEQ ID No. 2 or an analogue of said α-amylase, which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 2,

ii) reacts with an antibody raised against said α-amylase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said α-amylase.

7. The process according to claim 5, in which the parent α-amylase comprises the amino acid sequence of the *B. stearothermophilus* α-amylase shown in SEQ ID No. 4 or an analogue of said α-amylase, which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 4,

ii) reacts with an antibody raised against said α-amylase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said α-amylase.

8. The process according to claim 5, in which the parent α-amylase comprises the amino acid sequence of the *B. amyloliquefaciens* α-amylase shown in SEQ ID No. 6 or an analogue of said α-amylase, which

i) is at least 60% homologous with the sequence shown in SEQ ID No. 6,

ii) reacts with an antibody raised against said α-amylase, and/or

iii) is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding said α-amylase.

9. The process according to claim 3, in which the parent α-amylase is derived from a strain of an *Aspergillus* sp. such as *A. oryzae*, or *A. niger*.

10. The process according to any of the preceding claims, in which one or more methionine residues of the parent α-amylase has been replaced with a Leu, Thr, Ala, Gly, Ser, Ile or Asp.

11. The process according to claim 6, in which the methionine residue in position 197 of the parent α-amylase has been replaced with another amino acid residue different from Cys or Met.

12. The process according to claim 7, in which the methionine residue of position 200 and/or 206 has been replaced with another amino acid residue different from Cys or Met.

13. The process according to any of the preceding claims, in which further comprises addition of a stabilizer and/or a wetting agent.

14. The process according to claim 1, in which the bleaching agent is hydrogen peroxide.

15. The process according to claim 13 or 14, in which the oxidation stable α-amylase is used in an amount of 1-10 g/l and/or the hydrogen peroxide is used in an amount of 1-50 g/l.

16. The process according to any of the preceding claims, in which the combined desizing and bleaching treatment is performed at a temperature in the range of 30-100°C and a pH in the range of 6.5-11.

17. The process according to any of the preceding claims, in which the oxidation stable α-amylase is used in an amount of 1-5 g/l, preferably 1-3 g/l.

18. A composition to be used in a simultaneous desizing and bleaching process comprising an oxidation stable α-amylase in combination with at least one further component selected from the group consisting of wetting agents, dispersing agents, sequestering agents and emulsifying agents.

19. The composition according to any of claims 18, wherein the oxidation stable α-amylase is as defined in any of claims 2-12.

20. The use of an oxidation stable α-amylase for simultaneous desizing and bleaching of a fabric comprising starch or starch derivatives.

21. The use of an oxidation stable α-amylase for simultaneous desizing and scouring of a fabric comprising starch or starch derivatives.

22. The use according to claim 20 or 21, in which the oxidation stable α-amylase is as defined in any of claims 2-12.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00371

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/28, C12N 15/56, D06L 1/14 // C11D 3/386
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, D06L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EDOC, WPI, SCISEARCH, BIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO, A1, 9418314 (GENENCOR INTERNATIONAL, INC.), 18 August 1994 (18.08.94), page 4, line 22 - page 5, line 7, the claims --	1-22
X	WO, A1, 9116423 (NOVO NORDISK A/S), 31 October 1991 (31.10.91), page 3, line 9 - line 19 --	1-22
X	WO, A2, 9100353 (GIST-BROCADES N.V.), 10 January 1991 (10.01.91), see example 11 and claim 1 --	1-22

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 1995	31-05-1995

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00371

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO, A1, 9402597 (NOVO NORDISK A/S), 3 February 1994 (03.02.94), page 1, line 23 - line 28 --	1-22
X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 260, No 11, June 1985, David A. Estell et al, "Engineering an Enzyme by Site-directed Mutagenesis to Be Resistant to Chemical Oxidation", page 6518 - page 6521, see page 6518, left column line 1-5; page 6520, right column line 15-20 -- --------	1-22

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/05/95

International application No.

PCT/DK 94/00371

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO-A1-	9418314	18/08/94	NONE		
WO-A1-	9116423	31/10/91	EP-A-	0528864	03/03/93
			US-A-	5208158	04/05/93
WO-A2-	9100353	10/01/91	AU-B-	638263	24/06/93
			AU-A-	5953890	17/01/91
			EP-A,A,A	0410498	30/01/91
			JP-T-	4500756	13/02/92
			US-A-	5364782	15/11/94
WO-A1-	9402597	03/02/94	NONE		

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 94/00371

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

According to rule 13.2, an international application shall relate to one invention only or a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions makes over prior art.
Such a link between all the subjects of claims 1-22 would be the exchange of methionine by any other amino acid but cysteine, to create an oxidation stable enzyme. This *a priori* allegation, hower, is not acceptable due to the state of the art revealed by Estell DA et al. "Engineering an Enzyme by Site-directed Mutagenesis to Be Resistant to Chemical Oxidation", J Biol Chem, v 260, 1985, where it is stated that methionine should be exchanged to another amino acid in order to create an oxidation stable enzyme. Accordingly, the application is regarded to comprise the following inventions:

Invention 1, claims 6 and 11 completely, claims 1-5,10 and 13-22 partially: A process and composition for simultaneously bleaching and desizing a size cotaining starch using an α-amylase derived from SEQ ID No.2. The enzyme has been prepared from the parent enzyme by replacing one or more metionine residues with any amino acid residue different from cysteine or methionine.

Invention 2, claims 7 and 12 completely, claims 1-5,10 and 13-22 partially: A process and composition for simultaneously bleaching and desizing a size containing starch using an α-amylase derived from SEQ ID No. 4. The enzyme has been prepared from the parent enzyme by replacing one or more metionine residues with any amino acid residue different from cysteine or methionine.

Invention 3, claim 8 completely, claims 1-5 and 10-22 partially: A process and composition for simultaneously bleaching and desizing a size containing starch using an α-amylase derived from SEQ ID No. 6. The enzyme has been prepared from the parent enzyme by replacing one or more metionine residues with any amino acid residue different from cysteine or methionine.

Invention 4, claim 9 completely, claims 1-3 and 10-22 partially: A process and composition for simultaneously bleaching and desizing a size containing starch using an α-amylase derived from Aspergillus sp. The enzyme has been prepared from the parent enzyme by replacing one or more metionine residues with any amino acid residue different from cysteine or methionine.
In spite of the non-unity all the claims have been included in the search.